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13. ABSTRACT *(Maximum 200 Words)*

The scientific rationale for this Idea Grant was to clarify whether modifiable, mainly nutritional, influence levels of IGF-1, IGFBP-3, $1,25(OH)_2$ vitamin D ($1,25(OH)_2D$), and 25(OH)vitamin D. High levels of IGF-1 and low $1,25(OH)_2D$ have been shown to be related to elevated risk of prostate cancer. Some dietary factors that hypothetically impact on these serological factors, including total energy intake, calcium intake and protein intake, have been associated with prostate cancer risk. In the current study, none of the hypothesized factors showed correlations with $1,25(OH)_2D$ in 630 men. These results do not support our original hypothesis that 1,25 vitamin D is an important mediator of risk of several dietary risk factors of prostate cancer. We were able to demonstrate that intake of protein and minerals influence IGF-1 levels moderately in 751 men, but men with high intakes of protein and minerals were not at higher risk of prostate cancer relative to men with low intakes of these. These findings suggest that the range of dietary influences on prostate cancer in generally well-fed populations is unlikely to have a major impact on risk of prostate cancer.

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FINAL REPORT

Introduction

The scientific rationale for this Idea Grant is to clarify modifiable, mainly nutritional, determinants of levels of insulin-like growth factor-1 (IGF-1), insulin-like growth factor binding protein-3 (IGFBP-3), 1,25$(OH)_2$ vitamin D (1,25$(OH)_2$D), and 25(OH)vitamin D (25(OH)D). High levels of IGF-1 and low 1,25$(OH)_2$D have been shown to be related to risk of prostate cancer (*1*). Some dietary factors that hypothetically impact on these serological factors, including total energy intake, calcium intake and protein intake, have been associated with prostate cancer risk. Since the initial proposal, additional studies supporting an association between calcium intake and prostate cancer risk (*2*), and between IGF-1 and prostate cancer risk (*3*), have been published. Interest in the areas of IGF and vitamin D and cancer have been increasing. Thus, a need to determine factors, particularly modifiable ones, that influence levels of these hormones clearly exists.

Report

Vitamin D: The tasks related to vitamin D have been completed. Specifically, 630 specimens were retrieved, thawed, and aliquotted from Massachusetts Male Aging Study serum samples that have been stored in freezers in the laboratory of Dr. Christopher Longcope at the University of Massachusetts, Worcester. The samples were then shipped by overnight courier to Dr. Bruce Hollis at the Medical University of South Carolina. Because a higher than expected number of samples with insufficient volume were found, 630 rather than the projected 900 samples were sent to the laboratories. The reduced number of specimens is not expected to adversely effect the conduct of the study appreciably, particularly because of the high quality control of the laboratories (the coefficients-of-variation (CV%) were 5.4% for 25(OH)D, 5.3% for 1,25$(OH)_2$D). The better than anticipated CV%s in both laboratories helps offset the reduced number of analyzable specimens because the power to detect correlations is determined both by the sample size and by the accuracy of the laboratory assay.

We have met our goals of the statistical analysis of dietary factors (e.g., calcium, phosphorus, fructose, animal protein) for prostate cancer in predicting concentrations of 1,25$(OH)_2$D. These were reported in the previous annual report. The mean values for 1,25$(OH)_2$D and 25(OH)D were 31.9 pg/ml and 24.1 ng/ml, respectively. These are well within the expected range. Due to a programming error, the results for the hypothesized factors' correlations with 1,25$(OH)_2$D from the previous annual report were slightly incorrect. The corrected results are presented below in this report (the differences were minor and the conclusions were the same). The correct Pearson partial correlation coefficients (adjusted for total energy) between the nutrients and 1,25$(OH)_2$D were as follows:

calcium, $r = -0.063$ ($P = 0.13$)
phosphorus, $r = -0.03$ ($P = 0.43$)
animal protein, $r = -0.004$ ($P = 0.91$)
fructose, $r = 0.057$ ($P = 0.17$).

In addition, we examined very high intakes of calcium and also did not find a lower level of 1,25$(OH)_2$D: for men consuming less than 1500 mg of calcium, mean 1,25$(OH)_2$D was 32.0 pg/ml; for calcium intakes 1500-1999 mg, the mean 1,25$(OH)_2$D was 31.7 pg/ml; and for those consuming >2000 mg of calcium, the mean 1,25$(OH)_2$D was 30.6 pg/ml.

We also examined whether these nutrients correlated significantly with the $1,25(OH)_2D/25(OH)D$ ratio. In analyses unadjusted for total energy intake, there were moderate sized correlations, as follows:

calcium, r = -0.20 (P <0.0001)
phosphorus, r = -0.18 (P ,0.0001)
animal protein, r = -0.14 (P = 0.0007)
fructose, r = -0.08 (P = 0.04).

For energy adjusted nutrients, these correlations were in the same direction but somewhat weaker. The correlations were driven in large part because of a positive correlation between these nutrients and 25(OH)D rather than an inverse correlation with $1,25(OH)_2D$. These probably have a positive correlation with 25(OH)D because among the main sources of calcium, phosphorus and animal protein are dairy products and fish, which are the main sources of dietary vitamin D.

Unfortunately, these results do not support our original hypothesis that $1,25(OH)_2D$ is an important mediator of risk of several dietary risk factors of prostate cancer. While for some factors (fructose and animal protein) the proposed link with $1,25(OH)_2D$ was more speculative, the lack of association with calcium and phosphorus was surprising, given that metabolic studies clearly show that these impact on $1,25(OH)_2D$ levels on a short-term basis. While measurement error in the diet questionnaire may have accounted for some attenuation of an association, we do not expect that the measurement error was so large as to have entirely missed an important association. One of the original rationale of our study was that using the same methodology, we observed a strong relationship between calcium intake and risk of advanced prostate cancer; if $1,25(OH)_2D$ was the mediator of this relationship, we would have expected to observe a strong correlation between calcium and $1,25(OH)_2D$. These results suggest that dietary manipulation of $1,25(OH)_2D$ may not be the most feasible approach to prevent prostate cancer. Because we did not identify significant determinants of $1,25(OH)_2D$, we cannot derive an empirical model of $1,25(OH)_2D$ to use predict prostate cancer in the Health Professionals Follow-Up Study as proposed in Task 5.

Insulin-like Growth Factors (IGFs):

Introduction

The second major focus of this grant besides vitamin D was to examine predictors of IGFs. Based on the literature review, we hypothesized that various factors could be related to IGF-1 and IGFBP-3, and their ratio. Nutritional factors are critical regulators of IGFs. The nutritional factors most clearly related to IGF-1 levels are energy and protein balance. In particular, undernutrition of either of these dramatically lowers IGF-1 levels. For example, fasting for 10 days causes a four-fold reduction in IGF-1, levels associated with GH deficiency. Overnutrition may increase IGF-1 somewhat, but clearly excess calories are not nearly as strong a stimulus as nutritional restriction is in decreasing IGF-1 levels. Short-term feeding studies of protein deprivation demonstrate a potent and independent role of protein in stimulating IGF-1. Deficiency of essential amino acids, in particular, has a severe depressing effect on IGF-1 levels. A high carbohydrate diet also increased IGF-1 levels relative to a high fat diet, possibly by maintaining sensitivity to GH.

While restriction of energy and protein has clear and strong effects on IGF-1 levels, it is not clear whether the balance of macronutrients and possibly some micronutrients in non-restricted free-living populations with easy access to a wide variety of foods influences IGF-1 levels. Some limited data suggests that increase in milk may increase IGF-1, though whether this increase is independent of milk being an excellent source of essential amino acids is unknown. Minerals may also be important in influencing IGF-1 levels. In particular, evidence suggests that deficiencies of potassium, magnesium and calcium may lower IGF-1 levels. Some limited evidence also suggests that zinc intake may independently increase IGF-1 levels. (Devine A, AJCN 1998;68:200-6). However, the best dietary sources of zinc are also good sources of protein rich is essential amino acids; thus, whether the role of zinc is truly independent remains unresolved.

The primary goal of the study is to examine which dietary factors predict levels of IGF-1 and IGFBP-3, which are risk factors for prostate cancer.

Methods

As we had reported previously, when the IGF assays were started, Dr. Pollak noted that the concentrations were abnormally low, and we concluded that there was degradation in the samples, which rendered them essentially unusable for IGF assays. This degradation did not affect the vitamin D samples. To achieve our aims, we used instead archived plasma samples from the Health Professionals Follow-Up Study, instead of samples from the Massachusetts Male Aging Study as initially proposed. In the Health Professionals Follow-Up Study, we have dietary information (using the same dietary instrument as the Massachusetts Male Aging Study) in 1986, 1990, and 1994. 16,000 archived blood samples were collected 1993-1994. We used dietary intakes in 1994, the ones closest in time to the blood analyses, for the correlational analyses.

In the sample, we had complete information for dietary and covariate data, and IGF-1 and IGFBP-3 data for 751 men. The average age of the men at the time the blood was drawn was 65 years (Standard deviation = 8.0).

Results

We first examined the relationship between total energy intake and macronutrients in relation to plasma IGF-1, IGFBP-3 and IGF-1/IGFBP-3. As shown in table 1, no associations were noted for total energy intake nor for energy-adjusted fat intake. A slight positive association was suggested between total carbohydrate intake and IGF-1 and IGF-1/IGFBP-3. Total protein intake was positively associated with plasma IGF-1, IGFBP-3 and IGF-1/IGFBP-3. Comparing the top versus the low quintiles, the difference in means was approximately 15% for IGF-1 and 10% for IGF-1/IGFBP-3.

We next examined the intake of minerals in relation to plasma IGF-1, IGFBP-3 and their ratio. Because previous evidence suggests that more than one mineral, or their overall balance, may be important, and many of the minerals tend to be correlated as they have common dietary sources, we first examined the relationship between overall mineral intake and IGF. Specifically, we added quintiles of each of potassium, zinc, magnesium, calcium, and phosphorus to form a score ranging from 5 to 25. Then, this score was divided into quintiles. As shown in table 2, overall mineral intake was positively associated with plasma IGF-1, IGFBP-3 and IGF-1/IGFBP-3. Comparing the top versus the low quintiles, the difference in means was

approximately 15% for IGF-1 and 10% for IGF-1/IGFBP-3. These differences were quite similar in magnitude as those for total protein intake.

Because sources of protein and minerals may overlap, these could confound each other; thus, we examined these in the same model. Although the results were somewhat attenuated, each remained highly significantly related to plasma IGF-1, IGFBP-3 and IGF-1/IGFBP-3. For example, in the unadjusted model, the difference in IGF-1 between high and low quintiles of protein was 27.8 in the unadjusted model and 18.1 when adjusted for mineral intake. For mineral intake, the difference was 31.1 in the unadjusted model and 24.2 when adjusted for protein intake. The joint effect of total protein and mineral intake is shown in figure 1. There was approximately a 25% difference in IGF-1 between those in the top quartiles both of protein and mineral intakes compared to those in the bottom quartiles of both.

To examine whether one or some of the individual minerals were more important than the others, we simultaneously modeled potassium, zinc, magnesium, calcium and phosphorus in relation to plasma IGF-1, IGFBP-3 and IGF-1/IGFBP-3. The strongest associations were with phosphorus intake, with P-values of <0.0001, 0.015, and 0.004 for plasma IGF-1, IGFBP-3 and IGF-1/IGFBP-3, respectively. The only other statistically significant association was between zinc intake and IGF-1/IGFBP-3 (P = 0.04). Of note, phosphorus tends to be correlated both with the other minerals and with protein, as meat is a good source of phosphorus. When protein was added to the model with the individual minerals, the confidence intervals tended to widen and few results were statistically significant. Thus, the strong results for phosphorus may not necessarily reflect a causal effect of phosphorus alone, but rather that this nutrient is a good indicator of overall protein and mineral intake.

We next examined the major food sources of these nutrients. First, we examined the major protein sources, fish, red meat, poultry and milk. When adjusted for each other, fish and milk were associated with higher IGF-1 and IGFBP-3, but not with a higher ratio (table 3). Only poultry intake was associated with a higher ratio. When adjusted for total protein and mineral intake, the association between IGF-1 and IGFBP-3 with fish remained, but that with milk did not.

Finally, we examined whether predictors of IGF-1 were associated with risk of prostate cancer. Based on our findings, we computed a "predicted IGF score" based on reported intakes of total protein and minerals (potassium, zinc, magnesium, calcium, and phosphorus) because these appeared to be by far the strongest dietary predictors of IGF-1 levels. For minerals, we added the quintiles of each to form a score from 5 to 25 and then formulated quintiles of the score. We then formed a score of protein and minerals, to form a score of 2 to 10. For example, a man in the low quintiles of both protein and minerals would get a score of 2, a man in the second lowest quintile of each would get a score of 4, and one in the highest quintile of each would get a score of 10. Table 4 shoes the age-adjusted and multivariate relative risks for total prostate cancer and sub-categories of "aggressive" or advanced prostate cancer in the Health Professionals Follow-Up Study in relation to this score. These are based on cases diagnosed from 1986 to 1998. In general, no association was observed. We had especially high power to detect an association with total cases (n = 2,481). These results do not support the notion that dietary related influences on the IGF axis have an appreciable impact on prostate cancer risk.

Discussion

We were able to demonstrate that dietary factors influence the IGF axis. The major effects appear to be related to total protein intake and adequate intake of a variety of minerals,

although it was not possible to distinguish the independent effects of the various minerals. The influence was rather moderate, although about a 25% difference in IGF-1 levels was predicted between the high and low intakes of protein and minerals combined. Using intakes of protein and minerals combined, we did not see any relationship to prostate cancer risk. These findings suggest that the range of dietary influences on prostate cancer in generally well-fed populations is unlikely to have a major impact on risk of prostate cancer. This does not necessarily rule out that more extreme dietary differences may not have an influence. For example, energy intake restriction could potentially reduce IGF-1 levels more. Also, lower intakes of protein could also potentially have a greater influence. The median for protein intake in the lowest quintile was 69 g/day, which is above recommended intakes. Thus, it is possible that IGF-1 levels could be further reduced at lower intakes of protein. However, it is probably unwise for most men to go much below this level for other health reasons. Likewise, deficiencies of minerals may have adverse health effects. For these reasons, although dietary factors do have some impact on the IGF axis, the impact within the "acceptable" range of dietary factors is unlikely to have an important effect on prostate cancer risk.

Overall, our results indicate that (1) dietary influences on the vitamin D and IGF axes, at least within the ranges observed within well-fed populations, are relatively moderate in magnitude; (2) these differences are unlikely to have an important impact on prostate cancer risk; and (3) dietary and nutritional factors that influence prostate cancer risk are likely to act through mechanisms other than the IGF and vitamin D axes. Influencing prostate cancer risk through the IGF-1 axis, if warranted, would probably have to rely on pharmacologic rather than nutritional approaches.

Key Research Accomplishments

- Intake of total protein and minerals (potassium, zinc, magnesium, calcium, and phosphorus) were associated with IGF-1 and IGF-1/IGFBP-3 ratio. At least within the dietary range in generally well-nourished populations, the influence of these factors on the IGF axis was relatively moderate in magnitude. Based on these results, we conclude that the mechanisms whereby nutritional factors impact on prostate cancer risk are unlikely to be primarily through the IGF axis.

- We have completed determinations for vitamin D metabolites and have conducted the primary analyses correlating nutritional factors to $1,25(OH)_2D$ levels. None of the hypothesized factors showed correlations with $1,25(OH)_2D$. Based on these results, we conclude that the mechanisms whereby nutritional factors impact on prostate cancer risk are unlikely to be primarily through the vitamin D axis.

Reportable Outcomes (at this time)

None.

Conclusions

The scientific rationale for this Idea Grant was to clarify whether modifiable, mainly nutritional, influence levels of IGF-1, IGFBP-3, $1,25(OH)_2$ vitamin D ($1,25(OH)_2D$), and 25(OH)vitamin D. High levels of IGF-1 and low $1,25(OH)_2D$ have been shown to be related to risk of prostate cancer. Some dietary factors that hypothetically impact on these serological factors, including total energy intake, calcium intake and protein intake, have been associated with prostate

cancer risk. Thus, a need to determine factors, particularly modifiable ones that impact on levels of these hormones clearly exists. In the current study, none of the hypothesized factors showed correlations with $1,25(OH)_2D$ in 630 men. These results do not support our original hypothesis that 1,25 vitamin D is an important mediator of risk of several dietary risk factors of prostate cancer.

We were able to demonstrate that dietary factors influence the IGF axis. The major effects appear to be related to total protein intake and adequate intake of a variety of minerals, although it was not possible to distinguish the independent effects of the various minerals. About a 25% difference in IGF-1 levels was predicted between the high and low intakes of protein and minerals combined. Using combined intakes of protein and minerals, we did not see any relationship to prostate cancer risk, i.e. men with high intakes of protein and minerals were not at higher risk of prostate cancer relative to men with low intakes of these. These findings suggest that the range of dietary influences on prostate cancer in generally well-fed populations is unlikely to have a major impact on risk of prostate cancer.

Overall, our results indicate that (1) dietary influences on the vitamin D and IGF axes, at least within the ranges observed within well-fed populations, are relatively moderate in magnitude; (2) these differences are unlikely to have an important influence on prostate cancer risk; and (3) dietary and nutritional factors that influence prostate cancer risk are likely to act through mechanisms other than the IGF and vitamin D axes. Future study of influencing prostate cancer risk through the IGF-1 axis, if warranted, would probably have to rely on pharmacologic rather than nutritional approaches.

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Table 1. Mean plasma levels of IGF-1, IGFBP-3, and IGF-1/IGFBP-3 molar ratio by intake of nutrients in quartiles

Nutrient[a]	Median Daily Intake	IGF-1 (ng/ml)	IGFBP-3 (ng/ml)	IGF-1/IGFBP-3
Protein (g)				
	69.1	176.6	3025	0.226
	79.5	180.7	3001	0.233
	85.9	185.2	3038	0.236
	93.8	190.8	3136	0.237
	107.1	204.4	3215	0.248
	p(trend)	(<0.0001)	(0.01)	(<0.0001)
Carbohydrate (g)				
	192.6	185.5	3167	0.228
	232.8	188.5	3046	0.241
	254.8	184.0	3072	0.234
	279.9	189.1	3017	0.244
	310.0	193.2	3126	0.240
	p(trend)	(0.13)	(0.97)	(0.04)
Fat (g)				
	47.8	192.9	3089	0.242
	58.4	189.0	3177	0.232
	66.7	185.2	3029	0.238
	74.0	190.2	3088	0.240
	84.0	183.8	3017	0.235
	p(trend)	(0.16)	(0.24)	(0.54)
Calories				
	129.5	189.5	3086	0.239
	162.5	185.2	3094	0.233
	194.5	185.9	3032	0.237
	228.6	187.5	3014	0.243
	289.0	186.2	3170	0.237
	p(trend)	(0.45)	(0.39)	(0.80)

a Protein, carbohydrate and fat are adjusted for total energy. All results are adjusted for age and laboratory run.

Table 2. Age-adjusted mean plasma levels of IGF-1, IGFBP-3, and IGF-1/IGFBP-3 molar ratio by intake of minerals

	Quintile[a]	IGF-1 (ng/ml)	IGFBP-3 (ng/ml)	IGF-1/IGFBP-3
Minerals				
	1	174.4	2951	0.229
	2	179.6	3064	0.228
	3	190.5	3187	0.232
	4	194.2	3100	0.244
	5	205.5	3178	0.251
	p(trend)	(<0.001)	(0.009)	(<0.001)

a Quintiles based on adding quintile (1-5) of each of potassium, magnesium, zinc, calcium and phosphorus, and dividing the total score into quintiles

Table 3. Changes in plasma IGF-1, IGFBP-3, and IGF-1/IGFBP-3 molar ratio for specified increment of item

			IGF-1 (ng/ml)	IGFBP-3 (ng/ml)	IGF-1/IGFBP-3
Fish	3 servings/wk	Age-adjusted[a]	7.5[b]	92[b]	0.002
		Multivariate[a]	6.2[b]	87[b]	0.001
Poultry	3 servings/wk	Age-adjusted	1.9	-33	0.006[b]
		Multivariate	0.3	-38	0.005
Red Meat	3 servings/wk	Age-adjusted	-1.3	-1	-0.001
		Multivariate	-1.5	-13	-0.001
Milk	1 serving/day	Age-adjusted	5.4[b]	64[b]	0.002
		Multivariate	0.5	40	-0.002

a Age-adjusted = Adjusted for age and laboratory run only;
Multivariate = Adjusted for age, laboratory run, protein and minerals

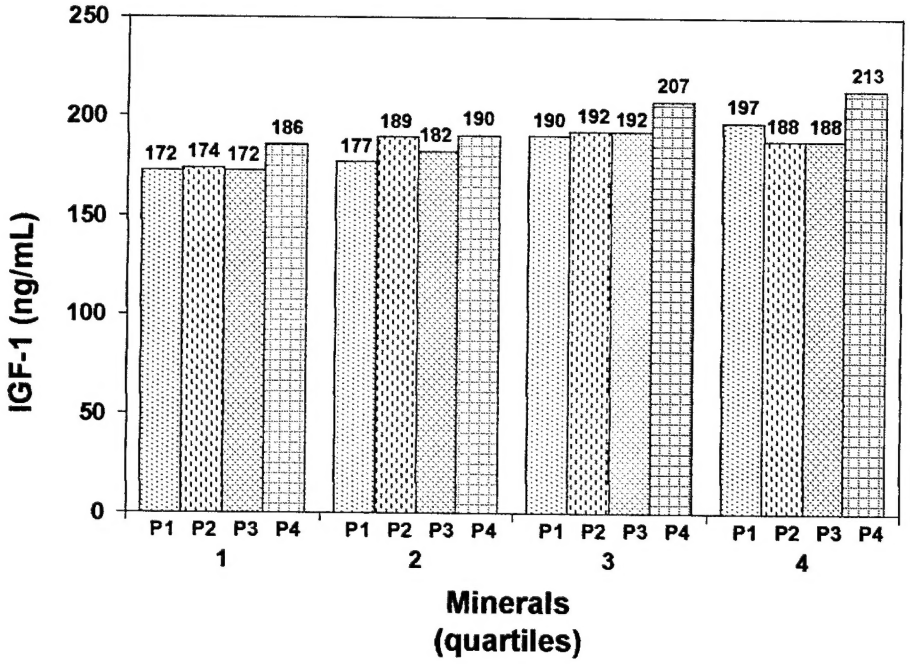
b $p < 0.05$

Table 4. Relative risk of total prostate cancer and some sub-categories of prostate cancer by IGF-1 Score (Protein quartiles plus mineral quartiles (2-10))

	IGF Score					
	2-3	**4-5**	**6**	**7-8**	**9-10**	**P trend**
Person Years	**44,253**	**125,319**	**70,404**	**134,089**	**91,136**	
Total (n=2481)						
Age-adjusted RR	1.0	1.10 (0.97, 1.25)	1.01 (0.87, 1.17)	1.04 (0.92, 1.18)	1.01 (0.88, 1.15)	0.80
Multivariate RR*	1.0	1.09 (0.96, 1.24)	0.99 (0.85, 1.14)	1.01 (0.89, 1.15)	0.99 (0.86, 1.13)	0.55
Organ-confined (n=1320)						
Age-adjusted RR	1.0	1.10 (0.93, 1.31)	0.99 (0.81, 1.21)	1.04 (0.88, 1.24)	1.00 (0.83, 1.20)	0.76
Multivariate RR*	1.0	1.08 (0.91, 1.28)	0.95 (0.77, 1.16)	0.99 (0.84, 1.18)	0.95 (0.79, 1.15)	0.35
Metastatic (n=278)						
Age-adjusted RR	1.0	1.21 (0.82, 1.77)	1.21 (0.78, 1.86)	1.20 (0.83, 1.75)	0.90 (0.59, 1.37)	0.61
Multivariate RR*	1.0	1.22 (0.93, 1.80)	1.22 (0.79, 1.88)	1.20 (0.82, 1.75)	0.91 (0.59, 1.39)	0.61
Fatal (n= 171)						
Age-adjusted RR	1.0	1.04 (0.65, 1.67)	1.01 (0.58, 1.74)	1.18 (0.75, 1.86)	0.65 (0.37, 1.12)	0.22
Multivariate RR*	1.0	1.08 (0.67, 1.75)	1.05 (0.60, 1.82)	1.22 (0.77, 1.93)	0.69 (0.39, 1.21)	0.31

* Multivariate RR adjusted for age, BMI, physical activity, total energy, calcium, lycopene, fructose, phosphorus, vitamin E and vitamin D

Figure 1.



P1 = Quartile 1 of protein intake, P2 = Quartile 2, etc.
Minerals = potassium, magnesium, zinc, calcium, phosphorus